Investigation of the cellular and soluble markers of inflammation for the assessment of cardiovascular risk in patients with acute coronary syndrome in Bangladesh

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Abstract: Acute coronary syndrome (ACS), one of the leading causes of mortality in Bangladesh, occurs when a plaque in the coronary artery suddenly ruptures and obstructs the flow of blood to the heart causing myocardial tissue necrosis. In this study, a total of 140 participants were enrolled including 70 ACS patients and 70 non-ACS controls. The total and differential WBC counts, myeloperoxidase (MPO) activity, ischemia-modified albumin (IMA), interleukin-6 (IL-6) and complement functions were investigated and the values were compared between the study groups. We found the total WBC count, neutrophil to lymphocyte ratio (NLR), plasma MPO activity, serum IMA, and IL-6 levels were significantly higher in the ACS patients while the complement-mediated bactericidal activity was significantly lower. The results on NLR, MPO activity, and IL-6 levels demonstrate activation of cellular and soluble inflammatory mediators in ACS, which may be potential diagnostic biomarkers.

Keywords: ACS; acute coronary syndrome; WBC counts; NLR; neutrophil to lymphocyte ratio; oxidative stress; MPO activity; markers of inflammation; IL-6; ischaemia-modified albumin; complement function; diagnostic biomarkers; Bangladesh.


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1 Introduction

Cardiovascular diseases (CVD) are responsible for approximately 30% of all deaths worldwide and more than half of these deaths are caused by coronary artery disease (CAD), thus, making it one of the leading causes of mortality (Mathers and Loncar, 2006). ACS is a subcategory of CVD which arises from CAD and is associated with a range of symptoms including reduced blood flow to the cardiac muscles altering the function of the heart. The sudden block in the coronary arteries supplying blood to the heart muscles lead to cardiac tissue necrosis which is presented as a myocardial infarction (MI). ACS encompasses three disorders of related etiology; namely ST-elevated myocardial infarction (STEMI), non ST-elevated myocardial infarction (NSTEMI) and unstable angina (UA). STEMI is associated with complete occlusion of the involved blood vessel whereas UA and NSTEMI are associated with partial blockage of the artery involved (DeWood et al., 1980; DeWood et al., 1986).

The underlying culprit of ACS is atherosclerosis, a complex, progressive and inflammatory condition of the arteries whereby lipoprotein and monocytes accumulate in the subendothelium to form a stenotic lesion known as a plaque. Plaque rupture activates platelets and stimulates the coagulation cascade leading to thrombus formation and blockage of blood flow to the myocardium, giving rise to ACS. Plaque disruption and endothelial erosion, the two main mechanisms that result in ACS are both consequences of enhanced inflammatory activity within the plaque (Ross, 1999). As a result, several components of the immune system play pivotal roles in the development of acute coronary syndrome.

Therefore, the atherosclerotic plaques are the site of a continuous inflammatory reaction. In fact, circulating neutrophils have been implicated in the pathogenesis of ACS. Increased expression of neutrophil adhesion molecules have been described in the peripheral blood of patients with ACS and several studies have indicated that neutrophils may promote plaque rupture through the release of proteolytic enzymes, arachidonic acid derivatives and superoxide radicals (Naruko et al., 2002; Roy et al., 2006). These components may go on to activate further leucocytes and generate a whole body immune response that can be observed in the circulation (Friedman et al., 1974). Thus, inflammatory responses in ACS include local inflammation in the vulnerable plaque and immune reactions associated within the thrombotic event itself as well as systemic immune activation.

Recently, the neutrophil to lymphocyte ratio (NLR) has been described as a prognostic marker in determining the outcomes of patients with ACS in which a high NLR has been found to significantly correlate with the risk of increased mortality.
in ACS patients (Bradley et al., 1982). Thus, NLR can be used as a simple biomarker that can provide robust information about the complex inflammatory activity in the local inflammatory site during the active phase of the syndrome. Additionally, myeloperoxidase (MPO), a neutrophil specific enzyme, characterised by powerful pro-oxidative and pro-inflammatory properties, is also secreted by activated neutrophils during inflammatory conditions (Bradley et al., 1982). New insights in the role of MPO have been emerging, describing it as a marker of risk for CAD and cardiovascular inflammation.

Several studies have shown both blood and leukocyte MPO activity to be higher in patients with CAD (Zhan et al., 2016). In addition, the reactive oxygen species (ROS) generated in the process may go on to disrupt the normal physiology in the cardiac and vascular tissues and may play an important role in cardiac pathophysiology (Sun, 2008). It is widely known that oxidative stress and inflammation go hand in hand. Furthermore, IL-6, a key player of inflammation is greatly elevated in CVD and studies have shown its role in altering vascular functions and NO and superoxide radical signalling (Schieffer et al., 2000). Additionally, oxidative stress has a profound effect on both circulating lipids and proteins, among which albumin is the most common. During ischemia, hypoxia or oxidative stress and free radical damage the N-terminal region of the albumin is modified such that it can no longer bind to certain metal ions (Christenson et al., 2001). This altered form of albumin is called ischemia modified albumin (IMA) and such a change of the structure of albumin can occur within minutes after an ischemic event, quickly becoming elevated in the bloodstream. As a result, IMA can also be studied as a predictor of myocardial damage.

It has been suggested that injured or necrotic myocardial tissue could serve as an activating source for the complement pathway (Iltumur et al., 2005). Several studies confirm a pivotal role of the complement system in CAD, demonstrating their presence in the intima of human atherosclerotic lesions as well as increased circulating levels of C3 and C4 (Vlaicu et al., 1985; Meuwissen et al., 2006). Since ACS is now being described as a systemic inflammatory disorder, roles of specific immune components, particularly those in the circulation, should be elucidated to gain further knowledge on the pathophysiology of the disease. Notably, risk factors for CVD and CAD vary according to geographic location but there are many evidences indicating the inordinate tendency of South Asians in developing CAD (Enas and Senthilkumar, 2005). In fact, the INTERHEART study, a large scale investigation, reported that the mean age of MI among Bangladeshis was 6 years lower than the Non-South Asians and lowest among all South Asians, thus placing Bangladesh in the highest risk category for development of CAD.

Although there is growing number of studies indicating the role of neutrophils, MPO and oxidative stress in the development of ACS, a complete picture of their mode of action remains to be elucidated. Additionally, although studies confirm the role of complement components in progression ACS, no studies have been conducted to determine whether the complement function is impaired among ACS patients. Thus, we conducted this study to investigate the leukocyte count and NLR values following development of ACS and compare them with a non-ACS control group. Furthermore, the plasma MPO activity, serum IL-6 levels and serum IMA activity were also compared among ACS patients and the control group in the hope of establishing a new ACS biomarker that can be reliably tested in the Bangladeshi population. Finally, complement mediated bactericidal activity was investigated in the ACS patients and compared to the
non-ACS group to determine whether there is any impairment in the immune function of ACS patients.

2 Methods

2.1 Study subjects

A total of 140 participants were enrolled in this cross-sectional study. These include 70 patients suffering from acute coronary syndrome (ACS), admitted in the coronary care unit (CCU) and progressive coronary care unit (PCCU) of the Cardiology Ward of the Dhaka Medical College Hospital. The diagnosis of ACS was done by expert physicians through the examination of characteristic electrocardiogram and biochemical changes. Exclusion criteria included those suffering from infections, impaired renal and liver functions, autoimmune disease and any other chronic inflammatory conditions. A further 70 subjects, employees of different offices, were enrolled as the control group. The control subjects did not have any prior history of diabetes or CVD. Both groups were thoroughly informed about the objectives of the study and full consent was obtained from all study participants prior to data collection.

2.2 Blood sample collection

Approximately 10 mL of peripheral venous blood was collected from each study participant with the help of an expert technician and divided equally in a purple capped dipotassium ethylenediaminetetraacetic acid (EDTA) containing tube for plasma collection and a screw capped glass tube for serum collection. The serum and plasma were collected in small aliquots in eppendorf tubes and stored at –20ºC. Blood smear slides were also prepared with fresh blood for each participant.

2.3 Determination of white blood cell counts

The total WBC count was determined for each blood sample using an improved Neubauer hemocytometer under the ×10 Objective of an Olympus microscope. For the differential count, Giemsa’s stain was used to stain the blood smear on the slides. The ×40 Objective of the Olympus microscope was used to visualise the different white blood cells. The results from the differential WBC count were used to determine the NLR for each sample.

2.4 Assay of plasma MPO activity

The plasma MPO activity was measured using the method described by Bradley et al. (1982). Briefly, an aliquot of 100 µL of 0.015% H$_2$O$_2$ was added to 2.9 mL of 0.167 mg/mL ortho-dianisidine (Alfa Aesar) in potassium phosphate buffer (final concentration of H$_2$O$_2$ was 0.0005%). Then 100 µL of test plasma was added to start the reaction. The change in absorbance at 460 nm was followed for 2 minutes using a Genesys spectrophotometer. The final result was expressed in Units/mg of total protein in the plasma sample where 1 unit of MPO was defined as the amount needed to degrade 1 µmole of H$_2$O$_2$ per minute at 25ºC.
2.5 Assay of serum IMA level

The ischemia modified albumin (IMA) in the serum was determined according to the method of Bar-Or et al. (2001). An aliquot of 60 µL of test serum was added to 400 µL of 45 mg/L cobalt dichloride in the Tris buffer and the reaction mixture was incubated for 10 minutes to allow cobalt binding to albumin. Then 200 µL of 1.5 mM dithiothreitol (DTT) prepared in Tris buffer was added to the mixture and further incubated for 2 minutes. Finally, 1.0 mL of 0.9% NaCl was added to quench the reaction and the absorbance was measured at 470 nm. A blank was prepared similarly with the exclusion of DTT to obtain serum background readings.

2.6 Determination of serum IL-6

Serum IL-6 levels of ACS and control subjects were measured using a Quantikine ELISA Kit (Human IL-6 immunoassay, Cat. no. D6050), according to the manufacturer’s recommended protocol (R&D Systems, Inc., Minneapolis, MN, USA).

2.7 Assay of complement mediated bactericidal activity

The complement mediated bactericidal activity was performed according to the protocol established by Islam et al. (2012). Escherichia coli DH5α were grown in nutrient broth for 15 h at 37°C; the bacterial cells were harvested, washed two times using excess of phosphate-buffered saline (PBS) and then adjusted to 0.600 OD at 620 nm. Immediately, aliquots of 200 µL of the bacterial cell suspensions (BCS) were mixed with 20 µL of serum and incubated for 30 min at 37°C. At the end of incubation, the remaining viable cells were serially diluted with PBS to 1:10 000, and 20 µL was spread on each of three agar plates and incubated for 16 hours at 37°C. The number of colonies formed was counted and the mean value for each serum was taken from the readings of three plates. For the negative control experiments, 20 µL of PBS (medium) was added to the BCS instead of serum, incubated and then serially diluted with PBS to 1:50 000, and 20 µL was spread on each of three agar plates.

2.8 Statistical analyses

Data analyses were carried out using the Statistical Package for Social Sciences (SPSS, version 17.0 for Windows). The statistical methods used were Independent samples t-test for the comparison of two groups (the ACS patients and control subjects), correlation analyses and simple statistical analyses. The results were considered significant when p was < 0.05.

3 Results

3.1 Study participants

Of the ACS patients (N = 70), 48 (68.6%) presented with STEMI, 17 (24.2%) presented with NSTEMI and 5 (7.1%) were suffering from UA. The control subjects (N = 70) had no prior history of cardiovascular disease (CVD) or any other chronic inflammatory disorders.
3.2 Baseline characteristics of the study subjects

The mean ± SD age of the patient group was 53.7 ± 11.2 years, ranging from 21 to 80 years, whereas the mean ± SD age of the control subjects was 45.5 ± 10.0 years, ranging from 21 to 75 years. The mean ± SD body mass index (BMI) of the ACS group was 23.3 ± 2.6 kg/m², which was not significantly different from that of the control group, 24.5 ± 3.8 kg/m². The mean number of days between hospital admission and blood collection of the patient group was 2.46 ± 1.2 with a minimum of 1 day and a maximum of 5 days interval. The mean duration of chest pain experienced by the patients was 15.5 ± 19.9 hours ranging from 1 to 72 hours. The troponin I value of the patients, measured upon diagnosis, was recorded and the mean was 6.7 ± 13.9 ng/mL, ranging from 0.02 to 54 ng/mL.

The mean systolic blood pressure (SBP) of ACS patient group was 129.1 ± 21.5 mmHg and diastolic blood pressure (DBP) was 83.8 ± 14.8 mmHg at the time of blood collection. Their mean pulse was 80.7 ± 12.9 bpm. Conversely, the control group had a mean SBP of 121.2 ± 7.0 mmHg and DBP of 79.6 ± 7.1 mmHg, and their mean pulse was 77.0 ± 7.2 bpm. Upon comparison of these data between the patient and control groups, the SBP was significantly higher in the patients (p < 0.05) but the pulse and DBP did not vary significantly.

3.3 Total WBC count

In the ACS patients, the mean WBC count was 9.74 ± 2.72 × 10⁹ cells/L and the values ranged from 4.5 × 10⁹ to 18.6 × 10⁹ cells/L. Furthermore, about 19% of the ACS patients had a WBC count that was higher than the upper limit (11.0 × 10⁹ cells/L) of the normal range. The mean WBC count among the control subjects was 7.95 ± 1.63 × 10⁹ cells/L and the counts fell in the range of 4.5 × 10⁹ to 11.0 × 10⁹ cells/L. These results showed significantly higher WBC counts in the ACS patients compared to control subjects (p < 0.001, Figure 1).

**Figure 1** Comparison of the total WBC count between the study groups. The ACS patients had significantly higher total WBC counts than the controls (p < 0.001) (see online version for colours)
3.4 Differential WBC count

In the control subjects, the mean neutrophil count was found to be $54.3 \pm 6.1\%$ while the lymphocyte count was found to be $40.9 \pm 5.6\%$. Their mean monocyte count was $2.5 \pm 1.2\%$, eosinophil count was $1.3 \pm 0.8\%$ and the mean basophil count was $1.0 \pm 0.8\%$. Compared to the controls, the neutrophil count in the ACS patients had a significantly higher ($p < 0.001$) mean value of $63.4 \pm 8.0\%$ while the lymphocyte counts were significantly lower ($p < 0.001$) with a mean value of $31.8 \pm 7.7\%$. Their mean monocyte count was $2.8 \pm 1.1\%$, eosinophil count was $1.5 \pm 1.5\%$ and the mean basophil count was $0.4 \pm 0.6\%$. The basophil count was significantly lower ($p < 0.001$) in the ACS patients but the monocyte and eosinophil counts did not vary significantly between the two study groups.

3.5 Neutrophil to lymphocyte ratio

The neutrophil to lymphocyte ratio (NLR) was calculated in the study subjects. The mean NLR among the patient group was $2.2 \pm 0.13$ and the values ranged from $1.09–7.42$. In contrast, within the control group, the mean NLR was $1.37 \pm 0.06$ and the values fell in the range $0.71–2.61$. Statistical analysis revealed that NLR values were significantly higher ($p < 0.001$) in the patients than in the controls.

Further analysis revealed that 72.7% of the control group had NLR values less than 1.5, 24.2% had values between 1.5–2.5 and only 3% had values greater than 2.5. This distribution was remarkably different in the ACS group where 24.6% had NLR values below 1.5 while 44.3% had NLR values between 1.5–2.5 and a striking 31.3% had values greater than 2.5. These findings have been presented in Figure 2. The NLR values were also compared between the different subgroups of ACS patients. The mean values of NLR for STEMI patients were $2.26 \pm 0.17$, NSTEMI patients was $2.18 \pm 0.21$ and for the UA group, the value was $1.66 \pm 0.23$. There was no significant variation in NLR between these subgroups.

Figure 2 Distribution of NLR values within the patient and control groups. Among the controls, 72.7% had NLR below 1.5, 24.2% had values in the range 1.5–2.5 and only 3% had values greater than 2.5. In the ACS group, 24.6% had NLR below 1.5, 44.3% had values in the range 1.5–2.5 and 31.3% had values greater than 2.5 (see online version for colours)
3.6 Plasma MPO activity

The mean MPO activity in the ACS patients was 17.04 ± 4.72 U/mg plasma proteins, ranging from 7.38–35.52 U/mg. Among the controls, the mean MPO activity was 9.89 ± 3.07 U/mg and the values varied from 3.27–18.09 U/mg. Statistical analysis revealed that the ACS patients had significantly higher MPO activities than the control subjects ($p < 0.001$, Figure 3). Further, the mean MPO activity in the STEMI group was 17.01 ± 5.11 U/mg, NSTEMI group was 16.73 ± 4.18 U/mg, and in the UA group was 18.42 ± 2.45 U/mg. Although the MPO activity in the UA group was higher, it was not significantly different from the other groups.

![Figure 3](image)

**Figure 3** Comparison of MPO activity between the control subjects and ACS patients. The box plots describe the quartiles of MPO activity, expressed in U/mg protein. The MPO activity was significantly higher in the ACS patients ($p < 0.001$) (see online version for colours)

3.7 Serum IMA levels

Ischemia modified albumin (IMA), a potent marker of cardiovascular oxidative stress, was analysed in the serum of the study subjects and compared. In the ACS patients, the mean ± SD of IMA was 2.11 ± 0.08 U/mL while the value in the control group was 1.38 ± 0.06 U/mL. The mean serum IMA was significantly higher in the ACS subjects compared to the controls ($p < 0.001$, Figure 4). Further, statistical analysis showed no significant variation between the mean IMA values among the ACS subgroups.

3.8 Serum IL-6 levels

It was found that the mean serum IL-6 level in the ACS group was 24.9 ± 30.7 pg/mL, with a minimum of 0.4 pg/mL and a maximum of 143.7 pg/mL. In the control group, the mean IL-6 level was 2.0 ± 3.1 pg/mL, ranging from 0 to 12.47 pg/mL. Upon statistical analysis, IL-6 level was found significantly higher in ACS group compared to the control subjects ($p < 0.05$).
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Figure 4  Comparison of ischemia modified albumin (IMA) values in the ACS patients and control subjects. The serum IMA in ACS patients was significantly higher than in the control subjects ($p < 0.001$) (see online version for colours)

3.9 Complement mediated bactericidal activity

The bactericidal activity of plasma complements was investigated in 35 randomly picked ACS patients and 35 randomly selected control subjects and the result was calculated as the percentage of bacterial cells killed. Among the ACS patients, the bactericidal activity varied from 91.5–100% with a mean value of 95.4 ± 2.2%. On the other hand, the bactericidal activity of the control subjects varied from 92.3–100% with a mean value of 96.7 ± 2.1%. The bactericidal activity was significantly lower in the ACS patients compared to the healthy control group ($p < 0.05$). These results are presented in Figure 5.

Figure 5  Scatter diagram for the complement mediated bactericidal activity in the study subjects. The mean bactericidal activity in the ACS patients, 95.4 ± 2.2%, was significantly lower than that in the control subjects, 96.7 ± 2.1% ($p < 0.05$) (see online version for colours)

3.10 Correlation of inflammatory markers in ACS

Pearson correlation analysis revealed a significant positive correlation between the total WBC count and IL-6 in the ACS patients ($p < 0.021$). Also, a significant positive correlation was found between NLR and IL-6 in the ACS patients ($p < 0.05$). Furthermore, there was a weak positive correlation between the neutrophil counts and MPO activity in the patients. A significant negative correlation was found between the plasma MPO activity and the complement mediated bactericidal activity in the ACS
patient group showing that the bactericidal activity decreased as the MPO activity increased \((r = -0.404, p < 0.02)\). This finding has been presented in Figure 6.

**Figure 6** A significant negative correlation between bactericidal activity and plasma MPO activity was found in the ACS patients \((p < 0.02)\) (see online version for colours)

### 4 Discussion

In view of ACS being described in the literature as a systemic inflammatory disorder, in this study certain biochemical and immunological markers of inflammation and oxidative stress as well as the immune function was investigated in the ACS patients and the findings were compared with non-CVD control subjects. Since diabetes mellitus (DM) is a strong risk factor for CAD and is also associated with inflammatory characteristic, patients with DM or any other chronic inflammatory condition has been excluded from the study to avoid false positive results.

In general, STEMI accounts for 70% of all MI while NSTEMI accounts for the other 30%. Interestingly, among the 70 ACS patients enrolled in this study, 68.6% presented with STEMI, 24.3% with NSTEMI and 7.1% with UA showing that the general statistics apply for even small sample sizes. However, in the USA, there has been an appreciable decrease in STEMI cases and an increase in NSTEMI cases (McManus et al., 2011) but there were no similar data available for Bangladeshi patients.

A specially designed questionnaire was used to obtain some general information of the ACS patients and the control subjects. It must be noted that the control subjects were not age-matched with the ACS patients and were slightly younger than the patients. In the current study, the systolic blood pressure was found to be significantly higher in the ACS patients \((p < 0.05)\) but the diastolic blood pressure, blood pulse and BMI did not vary significantly between the two study groups. However, it is important to note that the ACS patients enrolled were already hospitalised and being treated with anti-hypertensive drugs. Thus, the blood pressure at the time of blood collection may not reflect the blood pressure at the time of hospital admission or the MI event.

Although a few other studies (Myint et al., 2014) have shown the body mass index (BMI) to be a risk factor for CVD, no significant difference in the BMI of patients and control subjects was found in this study. However, it has been established that in Bangladesh, the central obesity, measured by the waist-to-hip ratio, is a better predictor of cardiovascular risk than peripheral obesity, which is measured by BMI (Sabah et al., 2014). The waist-to-hip ratio could not be measured in this study as some of the patients were critically ill.
One of the most important findings of the present study is the significantly higher count of leucocytes in the ACS patients compared to control subjects ($p < 0.001$). An elevated leucocyte count is a potent indicator of systemic inflammation and has been universally obtained in ACS patients (Pearson et al., 2003). The total WBC count has also been reported to have prognostic value and it is seen that an elevated WBC count during onset of ACS is associated with increased mortality and worse in-hospital outcomes. These studies have also led to the conclusion that greater predictive ability is contained within the differential WBC count than represented by the total WBC count alone. Several studies have pointed to the neutrophil count as having greater prognostic value leading to the investigation of neutrophil to lymphocyte ratio (NLR) (Friedman et al., 1974; Sweetnam et al., 1997).

NLR has been described as a potential marker to determine inflammation in cardiac disorders and predictor of long-term mortality in ACS patients. This study found significantly higher NLR values in ACS patients compared to that of the controls. Also the NLR values did not vary significantly between the ACS subgroups. These findings were consistent with those of a recent study (Zhan et al., 2016). The increase in neutrophil count may be due to a complex network of cytokines released prior to and during a cardiac event. Particularly, the key neutrophil production regulator, G-CSF has been reported to be elevated in patients diagnosed with ACS (Roberts, 2005; Leone et al., 2006) along with increased serum levels of IL-6 (Ikeda et al., 1992).

The present study found significantly higher levels of serum IL-6 in the ACS patients compared to the control subjects. Further, a significant positive correlation was found between the NLR and IL-6 levels in the patient group. Several cytokines including IL-3, IL-6, GM-CSF and G-CSF have been reported to be positive regulators of granulopoiesis and act at different stages of myeloid cell development; G-CSF has been found responsible for regeneration of cardiomyocytes through release of hematopoietic stem cells from the bone marrow into the peripheral blood circulation (Takano et al., 2003). The elevated total leucocyte count and neutrophilia may be side effects of the body’s natural reaction to remedy the cardiac event.

However, other studies report a more direct effect of neutrophils in the pathophysiology of ACS through the function of their specific enzyme, myeloperoxidase (MPO) (Shao et al., 2006). MPO is a good biomarker of neutrophil activation and cardiac inflammation. In the present study, the investigation of MPO activity in the circulation showed significantly higher levels ($p < 0.001$) in the ACS patients. No correlation was found between MPO and troponin I levels indicating that MPO activity is independent of myocardial damage but rather may play a role in plaque destabilisation prior to onset of ACS. These findings are in corroboration with other studies (Baldus et al., 2003). It is important to note, however, that the values used to express the circulating MPO are inconsistent in current literature and do not allow for direct comparison of data between researches. Additionally, we found a weak positive correlation between the neutrophil counts and MPO activity in the patient group only.

With the increase in inflammation, many studies have also demonstrated an increase in the levels of ischemia modified albumin (IMA) in the serum of ACS patients (Christenson et al., 2001). This N-terminal modified albumin arises as a result of oxidative stress and has been widely investigated as a potential biomarker for ACS. In this study, significantly higher levels of IMA were recorded in the patients compared to the controls ($p < 0.001$). These findings are in agreement with other studies conducted.
worldwide. However, an Indian study describes a synergistic relationship between troponin and IMA (Mehta et al., 2015), which was not found in this study.

There is considerable evidence suggesting that complement activation is continuously occurring in patients with ACS (Iltumur et al., 2005; Meuwissen et al., 2006). However, there has been no study on the function of the complement system following ACS. The complement system plays a key role in the bactericidal activity of plasma. The present study found that the ACS patients had significantly lower bactericidal activity than the controls \((p < 0.05)\). Furthermore, a significant negative correlation \((p < 0.02)\) was found between the bactericidal activity and MPO levels in the ACS patients suggesting that prolonged chronic inflammation in the body may subsequently impair complement function.

5 Conclusions
The results of this study demonstrate, there is indeed activation of the inflammatory pathways during ACS as shown by elevated levels of leucocytes, particularly neutrophils, and increased MPO activity and serum IL-6 levels in the circulation. Furthermore, ACS patients exhibit greater signs of oxidative stress compared to control subjects of similar age and also show impaired complement function. Finally, a negative correlation of MPO activity with decreased complement function suggest that chronic inflammation may lead to gradual loss of immunological function in the ACS patients.

Authors’ contributions
LNI conceived and designed the experiments. TZC performed all the experiments and data analysis. TZC and MK collected the samples. TZC and LNI participated in data interpretation and wrote the manuscript. MK critically reviewed the manuscript. All authors read and approved the final manuscript.

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